I

THE CHEMOTHERAPY OF SULPHONAMIDES *

By G. A. H. BUTTLE, M.A. (Camb.), M.R.C.S. (Eng.).

Introduction

The chemotherapy of bacterial diseases is a medical innovation of the last four years only. In the case of the protozoal infections, on the other hand, this form of treatment is comparatively ancient. The discovery of the effect of quinine in malaria dates from the seventeenth century, and mercury was used in syphilis in very early times. In 1910 Ehrlich and Hata published the discovery of the synthetic organic arsenical, Arsphenamine, and subsequently Neoarsphenamine, and their therapeutic effects in spirochætal infections. These were the first successful synthetic substances to be used in chemotherapy, and they have led to a most fruitful co-operation between the chemist and biologist which has given us several very important remedies.

The bacterial diseases had been considered to be resistant to this type of therapy. It was thought that these infecting agents were too primitive in structure to be influenced by any drug which was not noxious to the host; the only method of treating them was by the administration of sera, which combined with and neutralised the toxic products elaborated by the bacteria but did not directly influence their powers of multiplication.

The fact that the chemotherapy of protozoal diseases is so ancient may be attributed to the occurrence of quinine in the vegetable kingdom. Had there been a mineral or vegetable which contained sulphanilamide in some therapeutically active form we might have benefited by the potent action of this drug for the last 300 years.

* Based on an address delivered before the Medical Society for the Study of Venereal Diseases on January 27th, 1939.

Many attempts had been made to protect animals against bacterial infection. These however were all carried out with drugs which had been first tested as antiseptics *in vitro* and, owing to the very different conditions in and out of the body, they had met with little or no success. There was one exception—the discovery by Morgenroth and Levy in 1911 that ethylhydrocupreine would protect mice against small inocula of pneumococci ; this effect however was too slight to be of real clinical service.

Prontosil

The discovery of Prontosil, published in March, 1935, was due to the industry of the German biologist Domagk [1] and his chemical collaborators Meitzsch and Klarer. They had been trying the effect of a number of azo dyes on infected animals and noticed that the introduction of a sulphonamide group produced a substance which was therapeutically active in mice infected with streptococci, but did not act in any way as an antiseptic *in vitro*. This discovery was not published until some three years after it was made. In the intervening period some German clinicians had tried the new drug in a number of diseases. They found that it was active in a variety of streptococcal conditions, but thought that it was inactive in gonorrhœa.

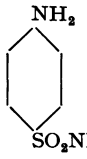
The German discovery was closely followed by the workers at the Pasteur Institute, and in December of the same year Tréfouël, Nitti and Bovet [2] found that the therapeutically important constituent of Prontosil was *p*-aminobenzene sulphonamide (a much simpler chemical than the original Prontosil). It produced equally good results in the protection of mice. This substance, which is now known as sulphanilamide, was originally described by the chemist Gelmo,[3] in 1908, but no practical use for it had been established in the ensuing twenty-seven years.

The first systematic clinical investigation of Prontosil was that of Colebrook and Kenny,[4] who treated 64 cases of puerperal sepsis with only three deaths, and these mainly due to causes outside influence of the treatment. Numerous other excellent papers on the clinical treatment of streptococcal diseases have since been published.

Sulphanilamide Compounds

Since the introduction of sulphanilamide a great number of substances (most, but not all, related to sulphanilamide) have been used in the chemotherapy of bacterial infections. From a chemical point of view, the

Fig. 1.
Sulphanilamide.



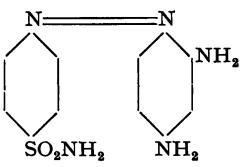
Amino group.
(Class I. substituents).

Amide group.
(Class II. substituents).

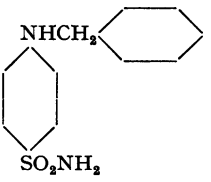
compounds of sulphanilamide (Fig. 1) can be divided into two classes, the first where there is substitution of the amino group (Fig. 2), the second when there is substitution of the amide group (Fig. 3). In the first class (Fig. 1)

Fig. 2.—Compounds of Sulphanilamide.
Class I. (*Substituents of Amino Group*).

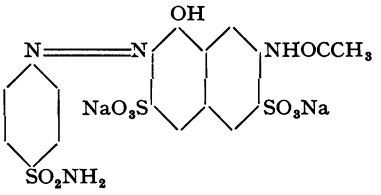
Prontosil.



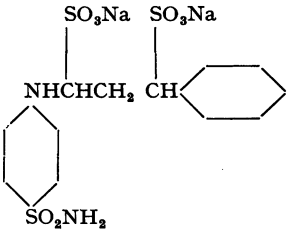
Proseptasine.



Prontosil Soluble.



Soluseptasine.



we have the original Prontosil, which is now used on an extensive scale only on the Continent. Work in England, America and France seems to show that this compound owed its activity to reduction in the body to active sulphanilamide and inactive triaminobenzene ; this view is, however, disputed in Germany by Domagk himself,

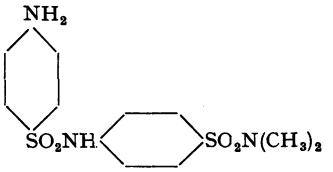
who considers that the sulphanilamide liberated is insufficient to explain the activity.

Proseptasine (benzylsulphanilamide) is another compound of this class which is still widely used in this country. It is non-toxic to laboratory animals. It was thought for a long time that this compound could not produce its therapeutic effect through its conversion into sulphanilamide in the body, but recently an American author [5] has adduced evidence to show that this conversion is responsible for the action in the mouse. The

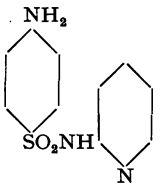
FIG. 3.—COMPOUNDS OF SULPHANILAMIDE.

Class II. (*Substituents of Amide Group*).

Uleron.



MB 693.



concentration of sulphanilamide liberated in the blood of the mouse after administration of Proseptasine is never greater than 4 mg. per cent., *i.e.*, about one-tenth of that which can be obtained by giving sulphanilamide itself. The therapeutic action of Proseptasine can be accurately imitated by very small doses of sulphanilamide adjusted so as to maintain a blood concentration of this order. In man and the monkey sulphanilamide can be readily detected in the urine when Proseptasine is given by mouth, but there is no detectable quantity in the blood. This seems to indicate that man and the monkey are less able to break down the compound than the mouse. If the increase in bactericidal power of the blood is determined in the case of these two animals after administration of a dose by mouth, one finds that the effect is either absent or very much smaller than after the administration of

sulphanilamide. The compound seems to be useful in mild streptococcal and, according to one report, in meningococcal infections in man, but it is not to be recommended for severe infections or those which are relatively resistant, where a high level of sulphanilamide in the blood is required. Similarly its activity is low in gonorrhœa where a high sulphanilamide level is wanted, not owing to the insensitivity of the infecting organism or the severity of the infection, but because of the relatively avascular nature of the tissues where the gonococcus has to be dealt with. Proseptasine has the advantage that an accidental overdosage would not cause serious symptoms as in the case of sulphanilamide ; also the toxic symptoms are less than those of equivalent quantities of the former drug. In a report [6] where a comparison of the drugs was attempted in erysipelas, Proseptasine has proved definitely but slightly less active than sulphanilamide.

The two soluble compounds at present in general use, Prontosil Soluble and Soluseptasine, both belong to this class of substances. They are of approximately the same activity, but Soluseptasine has the advantage of being colourless. There seems to be no advantage in giving these drugs by the parenteral route if sulphanilamide or MB 693 can be tolerated by mouth ; in cases where vomiting is severe, however, it is an advantage to have a soluble compound.

All the compounds of this class in which substituents are introduced into the amino group seem to owe their activity to their splitting up into sulphanilamide itself in the body. As might be expected, therefore, their range of activity is not greater, and in some cases is not as great, as that of sulphanilamide.

The second class of compounds (Fig. 3), on the other hand (where substitution takes place in the amide group), do not split up into sulphanilamide in the body, and in one case the range of activity is definitely larger than that of sulphanilamide. The two substances of this class which have been widely used are Uleron and MB 693. Domagk [7] claimed that Uleron was superior to sulphanilamide in staphylococcal and gas gangrene mouse infections, and it is widely used in Germany by clinicians for gonorrhœa. The claim that it is more active than sulphanilamide in staphylococcal infections has not been substan-

tiated by other workers. In clinical practice, if large doses are employed, it has the disadvantage of sometimes causing peripheral neuritis, a condition which sulphanilamide itself does not produce. If it is given in a series of short courses, however, as now recommended by the makers, this peripheral neuritis does not occur, and the compound is preferred to sulphanilamide by some authorities owing to its lower toxicity. It is said that Uleron is more effective in gonorrhœa if treatment is delayed for three weeks after the onset of the disease.

MB 693 (2-sulphanilylaminopyridine), recently described by Whitby,[8] is an advance in chemotherapy with these compounds. It is active against many strains of pneumococci in the mouse, whereas sulphanilamide is active only to some extent against Type III. Clinical results so far published have corroborated this observation, but there is a great variability in the sensitivity of different strains of pneumococci to the drug. These differences are not correlated with previously established serological groups, and no other known factor seems to be associated with them ; they appear to be a very profitable field for further investigation.

In our own experiments, MB 693 appears to have an advantage over sulphanilamide in pneumococcal infections which is more definite in the case of some strains than in others ; with some indeed there is little difference between the drugs.

Resistant strains of hæmolytic streptococci do also occur. We have found one belonging to Group B from a case of malignant endocarditis which was resistant to both sulphanilamide and MB 693, and strains of Group D., which usually occur in dogs, are generally resistant. Anaerobic streptococci also are completely resistant.

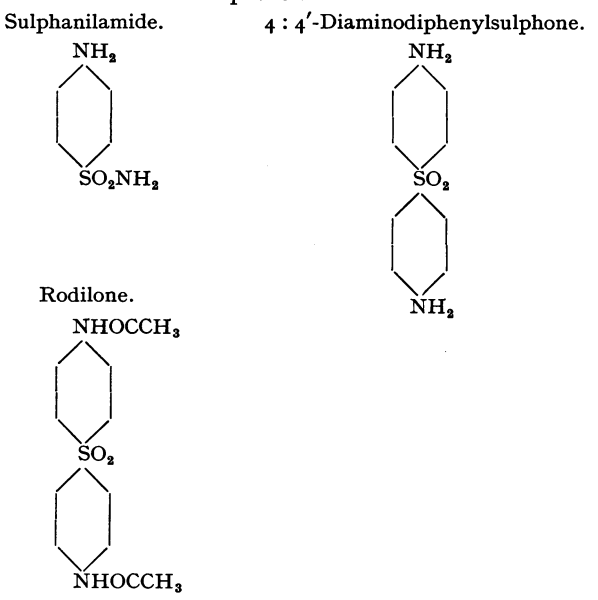
MB 693 also appears to be slightly more active than sulphanilamide in staphylococcal infections of mice ; however, the difference in this case is not very great. In streptococcal infections of mice it is at least as active as sulphanilamide ; our own experiments do not entirely confirm Whitby's findings that it is five times as active, but it seems likely that this difference may be due to the absorption difficulties to which I shall refer later. It seems to be the most active substance in gonorrhœa. The only disadvantages are that it causes more vomiting than sulphanilamide, and it is comparatively costly.

Sulphone Compounds

In addition to sulphanilamide and its compounds, there is another series of substances which exert the same type of action. 4 : 4′-diaminodiphenylsulphone (Fig. 4) has a much more potent therapeutic action in infected mice than sulphanilamide, but although it is more active it is also more toxic, and it appears from the small number of trials which have so far been done that in clinical work

Fig. 4.

Sulphones.



this increase in toxicity more than offsets the increased activity. A number of compounds of lower toxicity have been prepared by substitution of the amino groups in this substance, but it seems that these owe their activity to the slow liberation of the parent substance in the body, so that they do not give us very much reduction of toxicity without impairment of activity. One of them, the diacetyl derivative, is marketed under the name of Rodilone. It is of very low toxicity, but owing probably to the slow liberation of the parent substance it appears to be a little less active than sulphanilamide. Although smaller doses of drug are required than in the case of

sulphanilamide, it will not deal with severe infections even if larger doses are given.

It appears that, however much Rodilone is given, only a limited amount of the active substance can be produced in the animal. It is possibly a better fool-proof drug than Proseptasine as the minimum effective dose is much smaller, also it has some action in gonorrhœa whereas the action of Proseptasine is only slight. It has not been used widely in this country, but it would seem to have advantages if it is desired to produce a drug which will do no harm however much is taken.

Range of Activity in Bacterial Infections

Prontosil was at first thought to have a specific action on the streptococcus alone and to be incapable of influencing other infections, but later some English authors showed that sulphanilamide had a very potent therapeutic effect in meningococcal infections of mice.[9], [10] No. experiments were possible on gonococcal infections of animals at this time, but Levaditi and Vaisman [11] have

Fig. 5.—Protection of Mice against Gonococcal Infection.

Average survival time of 10 mice (10 days max.).

	Mgm.	Days.
Controls	*Nil*	2·5
Sulphanilamide	10	10·0
MB 693	10	8·6
Uleron	10	4·9
Rodilone (diacetylaminodiphenylsulphone)	10	3·5
Glucoside of diaminodiphenylsulphone	5	4·9

The drugs were given by mouth immediately after infection, and repeated six and twenty-one hours later.

recently been able to kill mice by using very large numbers of gonococci and have found that a large proportion of the animals can be saved by treating them with sulphanilamide and sulphone compounds. Fig. 5 illustrates an experiment done by Levaditi's method and using his strains of gonococci. The number of infecting

organisms is very large and the infection is of quite a different type from that found in clinical practice. It is rather surprising therefore that the results agree fairly well with those of the clinical assays of these compounds ; the exception in the case of MB 693 is probably due to the difficulty of absorption to which I shall refer later. Numerous excellent clinical assays of various compounds in gonococcal infections have been made ; but I will not refer to these, as members of the Society will be more familiar with them than I am, and I hope to know more about the question after the next meeting.

Sulphanilamide is now used in a great variety of conditions. It has been shown to be very effective in urinary infections with *B. coli* and *B. proteus* and there is a certain amount of evidence that gas gangrene and *Brucella abortus* infections, both experimental and clinical, are favourably influenced. There is experimental evidence that early treatment will influence typhoid infection in mice. Clinically the drug has been found to be effective in urinary carriers, but there is very little published about the treatment of the disease itself. Presumably treatment, to be effective, would have to be started in the first week, when the diagnosis is difficult.

Staphylococcal infections, both clinical and experimental, appear to be slightly influenced by sulphanilamide, and recent evidence shows that MB 693 is superior to sulphanilamide in experimental infection. Infections with this organism give rise to localised abscesses much more readily than do streptococcal infections, and in our experimental work we frequently find animals which appear to have been cured by treatment, but when a necropsy is made a month after infection there are a number of localised staphylococcal abscesses. The organisms in these abscesses are of course walled off from the influence of the drug. It may be mentioned that when gland abscesses occur in gonococcus cases these also seem to be resistant to chemotherapy.

It appears probable that these drugs have some degree of activity in all infections where death is brought about by a rapid multiplication of the invading bacteria ; they fail completely in infections where a small number of bacteria can elaborate a sufficiently potent toxin to kill the animal, as in tetanus and diphtheria. In these latter serum is the only agent which has any influence on the

infected animal, although in the test-tube the growth of *B. diphtheriæ* is inhibited quite as much by sulphonamide remedies as is that of streptococci.

It would appear that in infections where the rapid multiplication of bacteria is associated with the production of a potent toxin both chemotherapy and serum treatment should be applied together; examples of this are the gas gangrene infections and possibly pneumonia and scarlet fever.

Many more diseases may be found to belong to this group when the question of toxin production has received further investigation. To judge by recent press correspondence, the production of gonococcal antitoxin has met with questionable success, yet there seems to be evidence that a degree of immunity is produced by the disease itself. It may be of course that this immunity will not be conveyed to serum.

An example of this combined treatment is the finding of Lowenthal [12] that, if mice are infected with streptococci, simultaneous treatment with sulphanilamide and anti-streptococcus *type-specific* serum will save them at a late stage of the infection when either agent *alone* is ineffective.

The fact that the chemotherapy of gonococcal infections is more effective when it is started after the disease has been established for some weeks is another example of this; in the interval between infection and treatment some immunity against the organisms has developed.

Whilst a great variety of bacterial conditions appear to be amenable to treatment, the results with virus infections have been negative. There are two exceptions, lymphogranuloma inguinale and trachoma. In lymphogranuloma there is both experimental and clinical evidence of therapeutic effect; in trachoma only clinical evidence. In both cases there is some doubt as to whether the infecting organism can be classed as true virus.

Two other diseases have recently been said to be influenced by the drugs, plague and malaria. The evidence for the former rests on an experimental basis only, but in the case of the latter human infections with the benign tertian and subtertian varieties are said to be favourably influenced. While the drugs are ineffective against *Pl. relictum* in bird malaria, they are found to be effective against *Pl. knowlesi* in the monkey. At present

the value of the drug in malaria is hardly established, as reports are not unanimous.

There appears to be no specificity in the action of any of these drugs in bacterial infections. One drug is not better for one type of infection and another for another type, as in the case of the chemotherapy of protozoal disease when quinine is active in malaria and the arsenicals in spirochæte infection. These drugs are active in some degree on all bacteria; the more potent drugs act on a larger range of infections than the less potent ones.

Absorption and Excretion

An extensive pharmacological study of sulphanilamide has been carried out by Marshall and his colleagues.[13, 14, 15, 16, 17, 18, 19] They have shown that sulphanilamide is absorbed almost entirely from the small intestine and not from the stomach. The concentration in the blood rises rapidly after taking the drug by mouth and reaches a maximum in about three hours, thereafter gradually falling to zero in the course of twenty-four hours. The drug finds its way into the secretions of the body and into all tissues except bone and fat. The concentration in the body fluids, *e.g.*, cerebro-spinal fluid, is nearly as great as that in the blood. It is excreted in the urine partly as the free base and partly in the acetylated inactive form. It is interesting that the rate of elimination is independent of the plasma level but follows the urine flow. The explanation of this seems to be that 70 to 80 per cent. of the drug is re-absorbed in the kidney tubules after filtration through the glomeruli. This dependence of the rate of elimination on the urinary flow makes it possible to wash the drug out of the body by promoting diuresis when there has been overdosage or evidence of toxic symptoms.

MB 693 is absorbed slightly slower than sulphanilamide. It is excreted in the urine partly as the free base and partly as the acetyl derivative. Marshall [19] has found that the absorption of the free base is irregular; sometimes mice treated with large doses have lower blood concentrations than those treated with smaller ones. Absorption is greatly facilitated by giving the compound by mouth, together with sodium bicarbonate or HCl, or by using the very soluble sodium salt. It seems possible

that these results of Marshall may have important clinical applications in increasing the effect of the drug, but it should be mentioned that the toxicity of the substance as judged by blood concentration measurements is greater than that of sulphanilamide.

Toxicity

The acute toxic effects of these drugs in animals are confined to nervous symptoms—paralysis with incoordinate movements in the case of sulphanilamide and hyperexcitability in the case of the sulphones and MB 693.

In clinical work these symptoms are hardly ever observed, the doses used being proportionately much smaller ; a number of more chronic toxic symptoms have been seen. These are now well known, the most common being cyanosis, drug fever, lassitude, nausea, sulphæmoglobinæmia and methæmoglobinæmia. Agranulocytosis and hæmolytic anæmia are the most severe effects, but fortunately both are rare (only 15 cases of agranulocytosis have been reported). It occurs after prolonged therapy with large doses. Cases where there is a slight fall in the white cell count are however a relatively common occurrence. Hæmolytic anæmia which occurs in the first few days of treatment is also rare. Cyanosis seems to be partly due to the elaboration of pigments from condensation of products of the drug itself, and it is usually thought that it can be neglected from consideration as a symptom of overdose. Drug fever may give rise to confusion with a recrudescence of the infection, but it ceases when the drug is discontinued. Sulphæmoglobinæmia is due to the catalysis of the union of H_2S from the intestine with hæmoglobin by sulphanilamide. It is avoided by withholding purgatives and using a low residue diet excluding eggs.

MB 693 was originally thought to be less toxic to animals than sulphanilamide. Recently Marshall [19] has shown that this apparent lack of toxicity is due to deficient absorption. When given as the soluble sodium salt, which is much better absorbed, it is more toxic than sulphanilamide. Toxic symptoms (hyperexcitability) occur with lower blood concentrations than in the case of sulphanilamide.

In the dog a blood concentration of 35 mg. per cent. causes strychnine-like convulsions, whereas with sulphanilamide 50 mg. per cent. causes no noticeable symptoms. MB 693 has now been given to many patients and there have been few reports on toxic symptoms not produced by sulphanilamide ; it does, however, produce more nausea and vomiting. Perhaps it would be well to mention that, as sodium bicarbonate increases the absorption of the drug, gastric disturbances following its use may not be benefited by treatment with alkalis.

Mode of Action

When Prontosil was first discovered its mode of action was quite obscure ; although it had a potent therapeutic effect when given to infected animals, it had no effect on organisms *in vitro*. Domagk [1] found, however, that sections of mouse peritoneum, taken twenty-four hours after infection and treatment, showed active phagocytosis and a great reduction in the numbers of cocci as compared with the controls ; forty-eight hours after treatment there were no cocci to be seen in these sections.

After Tréfouël and his colleagues [2] suggested that the action of Prontosil depended on the liberation of sulphanilamide, some English authors [20] showed that this compound had an action *in vitro* in inhibiting the growth of streptococci. This effect was more marked in blood than in broth. In human and monkey blood some thousands of organisms per cubic centimetre were destroyed by the addition of small concentrations of sulphanilamide. Similarly it was shown that the administration of either sulphanilamide or Prontosil by mouth induced a very marked increase in the bactericidal power of the blood in patients suffering from streptococcal disease and in normal persons or monkeys. These results have been corroborated by workers in America and France, but are disputed in Germany.

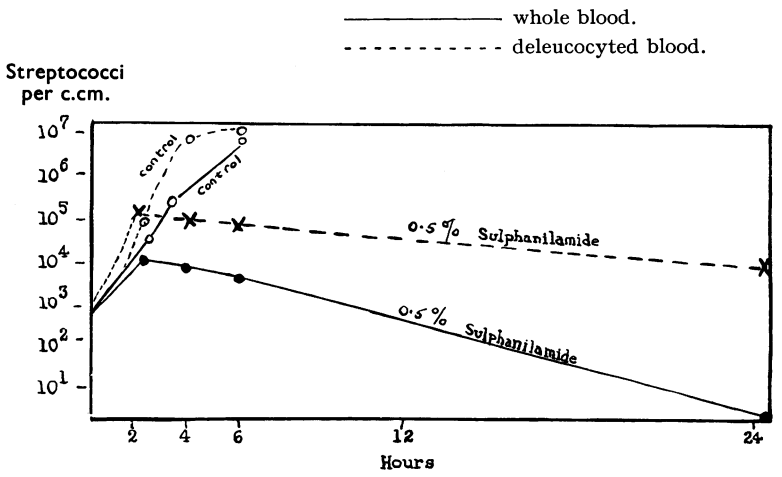
Fig. 6 shows the rate of growth of streptococci in samples of monkey blood with and without sulphanilamide, and also in similar samples of blood from which the leucocytes have been removed. For the first two hours the rate of growth with and without sulphanilamide is about the same ; subsequently the streptococci in the controls go on multiplying in a logarithmic manner,

whereas in the tubes containing sulphanilamide a gradual decrease in the streptococcal population takes place. The only difference between the normal blood and that from which the leucocytes have been removed is that in the latter case the outgrowth of streptococci is more rapid and their subsequent removal is much slower than when the leucocytes are present.

Fleming [21] has shown that in blood *in vitro* MB 693 is more effective than sulphanilamide on streptococci and pneumococci. With the latter organism complete destruction will not occur except in the presence of leucocytes.

Corroborative evidence for this theory of the mode of

FIG. 6.—EFFECT OF *p*-AMINOBENZENESULPHONAMIDE ON HÆMOLYTIC STREPTOCOCCI IN BLOOD.



action was obtained by Hoare [22] from the observation that two strains of streptococci from cases refractory to sulphanilamide treatment were insensitive to sulphanilamide *in vitro*, whereas all the others from the cases which responded were sensitive. McLean and others [23] have shown that strains of pneumococci which were not influenced by MB 693 *in vitro* produced mouse infections which were not amenable to treatment, whereas other strains which were sensitive *in vitro* produced infections in the mouse which could be successfully treated.

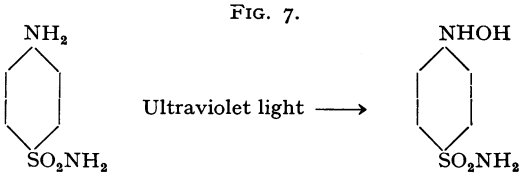
If a small quantity of peptone is present in these *in vitro* experiments with sulphanilamide the outgrowth of the streptococci is accelerated and their subsequent removal delayed. In fact, in the absence of leucocytes,

complete sterilisation does not usually occur. On the basis of this effect of peptone Lockwood [24] has put forward the idea that sulphanilamide may owe its action to the inhibition of the proteolytic enzymes of the bacteria. I do not think this explanation is necessarily supported by the experimental evidence, as the acceleration of growth due to peptone is seen in the controls as well as in the sulphanilamide specimens.

Evidently the drugs act on the bacteria in some way so as to reduce their powers of multiplication and in certain cases in time to exterminate them. This extermination depends on the presence of other factors either inhibiting or accelerating bacterial growth, *e.g.*, the leucocytes or the peptone.

As the effect of the drugs may well be described as an

FIG. 7.



NH_2OH inhibits catalase. This allows H_2O_2 formed during growth to accumulate within bacteria and gradually kills them.

antiseptic effect exerted *in vivo*, a comparison of sulphanilamide with other well-known antiseptics is perhaps not out of place. Thus the action of sulphanilamide *in vitro* is only to delay the rate of growth of the cocci and under certain conditions to kill them very slowly ; it does not kill large numbers of organisms rapidly like the ordinary antiseptic substances (phenol or the antiseptic dyes, for instance). Nevertheless, when given to infected animals the ordinary antiseptic substances have no effect, but sulphanilamide is very active. The properties which give sulphanilamide this potent action *in vivo* appear to be, firstly, that it is a substance which inhibits bacterial growth, but is not toxic to the animal and does not damage the leucocytes ; thus it allows the defensive cells to exert their full measure of activity in destroying the organisms which are deprived of their powers of multiplication. Secondly, it diffuses freely throughout the animal body and is not rapidly destroyed or removed as is the case with the ordinary antiseptic dyes.

The method by which the drug attacks the bacteria is

still quite an open question. There have been many suggestions that it acts on some bacterial enzyme. I have referred to one of these suggestions already. Other enzymes which have been the subject of experiment are the bacterial dehydrogenase, which is apparently uninfluenced by the drug, and the catalase, inhibition of which would cause bacterial destruction through the accumulation of peroxides (Fig. 7). Hydroxylamines are known to inhibit catalase, and it has been suggested that sulphanilamide might be converted into the corresponding hydroxylamine in the body, but no definite evidence of this has been produced. The inhibition of the bacterial catalase would allow peroxides to accumulate within the bacterial cell, thus destroying it from within. Shinn, Main and Mellon [25] have recently adduced evidence to show that peroxide accumulates more rapidly in cultures of pneumococci in the presence of sulphanilamide, but their experiments await confirmatory evidence.

Some observations have been made on the effect of these drugs on the capsules of organisms. Some years ago Levaditi [26], [27] found that the streptococci in the peritoneum of treated mice were uncapsulated, but so far this observation has not been repeated. Recently Whitby [28] has observed that the capsules of pneumococci in the peritoneum of mice treated by MB 693 became crenated and eventually disappeared.

Levaditi [29] has found that sulphanilamide exercises a protective action in the case of animals injected with bacterial endotoxins (*e.g.*, that of Flexner dysentery). On this basis he considers that it has an antitoxic as well as an anti-bacterial action. Our own experiments on mice injected with Flexner endotoxin do show that sulphanilamide treatment will delay the time of death slightly; our results, however, are not quite as conclusive as those of Levaditi, and at present we should not like to draw any conclusions from them.

To Summarise

MB 693 is more effective than sulphanilamide in pneumococcal infection. It is, however, slightly more toxic as judged by blood concentration measurements. The lack of toxicity in the original experiments has been shown to be due to lack of absorption. The absorption

of this drug is facilitated by simultaneous administration of alkalies. The drugs of the sulphonamide group appear to act as weak antiseptic substances acting *in vivo* without damaging the host.

REFERENCES

(1) DOMAGK, G. : *Dtsch. Med. Wschr.*, 1935, **61,** 250.
(2) TRÉFOUËL, J., et MME. J., NITTI, F., and BOVET, D. : *C. R. Soc. Biol. Paris*, 1935, **120,** 756.
(3) GELMO, P. : *J. Prakt. Chem.*, 1908, **77,** 369.
(4) COLEBROOK, L., and KENNY, M. : *Lancet*, 1936, *i.*, 1279.
(5) LOCKWOOD, J. S. : *J. of Pharm.* (in the Press), 1939.
(6) SNODGRASS, W. R., ANDERSON, T., and RENNIE, J. L. : *Brit. Med. J.*, 1938, **2,** 399.
(7) DOMAGK, G. : *Klin. Wschr.*, 1937, **16,** 1412.
(8) WHITBY, L. E. H. : *Lancet*, 1938, *i.*, 1210.
(9) BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D. : *Lancet*, 1936, *i.*, 1286.
(10) PROOM, H. : *Lancet*, 1937, *i.*, 16.
(11) LEVADITI, C., and VAISMAN, A. : *Pr. Méd.*, 1937, **45,** 1371.
(12) LOWENTHAL, H. : *Lancet*, 1939, *i.*, 197.
(13) MARSHALL, E. K., EMERSON, K., and CUTTING, W. C. : *J. Amer. Med. Ass.*, 1937, **108,** 953.
(14) MARSHALL, E. K., EMERSON, K., and CUTTING, W. C. : *J. Pharmacol. and Exper. Therap.*, 1937, **61,** 191.
(15) MARSHALL, E. K., EMERSON, K., and CUTTING, W. C. : *Ibid.*, 1937, p. 196.
(16) MARSHALL, E. K., EMERSON, K., and CUTTING, W. C. : *Science*, 1937, **85,** 202.
(17) MARSHALL, E. K. : *Proc. Soc. Exp. Biol and Med.*, 1937, **36,** 422.
(18) MARSHALL, E. K. : *J. Biol. Chem.*, 1937, **122,** 263.
(19) MARSHALL, E. K., BRATTON, A. C., and LITCHFIELD, J. T. : *Science*, 1938, **88,** 597.
(20) COLEBROOK, L., BUTTLE, G. A. H., and O'MEARA, R. A. Q. : *Lancet*, 1936, *ii.*, 1323.
(21) FLEMING, A. : *Lancet*, 1938, *ii.*, 74.
(22) HOARE, E. D. : *Lancet*, 1938, *i.*, 655.
(23) MACLEAN, I. H., ROGERS, K. B., and FLEMING, A., : *Lancet*, 1939, *i.*, 562.
(24) LOCKWOOD, J. S. : *Journ. of Immunol.*, 1938, **35,** 155.
(25) SHINN, L. E., MAIN, E. R., and MELLON, R. R. : *Proc. Soc. Exp. Biol. and Med.*, 1938, **39,** 591.
(26) LEVADITI, C., and VAISMAN, A. : *C. R. Soc. Biol.*, 1935, **119,** 946.
(27) LEVADITI, C. : *Ibid.*, 1935, **120,** 1077.
(28) WHITBY, L. E. H. : *Lancet*, 1938, *i.*, 1210.
(29) LEVADITI, C., and VAISMAN, A. : *C. R. Soc. Biol.*, 1938, **127,** 463.